TES07 - Microspectrophotometry for Fiber Color Analysis

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Scope

This document addresses the performance monitoring of the microspectrophotometer (MSP) systems. Color measurement of fibers is conducted using the MSP systems identified below. In order to ensure the instrument is operating within expected parameters, the five standards listed below must be analyzed each day the instrument is used.

This document also addresses the procedures by which dyed fibers are analyzed utilizing the MSP.

Safety Precautions

Universal precautions will be followed. No specific hazards are associated with the microscopic techniques performed.

Materials Required

- Craic QDI 1000 or Craic QDI 2010 microspectrophotometer
- Holmium oxide standard and certificate (National Institute of Standards and Technology [NIST] traceable)
- Didymium standard and certificate (NIST traceable)
- Neutral density standards (three) with the following optical densities:
  - 0.1
  - 0.5
  - 1.0

Standards and Controls

The holmium oxide, didymium and the three (3) neutral density standards are analyzed to assess daily operating performance and integrity of the system. The standards used for this procedure are NIST traceable, and therefore have a finite time frame in which they can be used. Ensure that the standard set being used is within the listed time frame on the certificate associated with that set.

Procedure

If two fibers cannot be differentiated utilizing the characteristics listed in TES04 Forensic Fiber Examinations, and if the fiber has been dyed, the fibers must be analyzed utilizing MSP. The use of the MSP may be necessary even if it appears that the fibers being compared have not been dyed.

Daily Calibration for the QDI 1000 microspectrophotometer

Verification of calibration must be performed each day the MSP system is used. Save all of the spectra in an appropriate folder on the computer. Spectral files should be stored in each examiner's folder which contains a folder with the MPD Laboratory number as the folder's name.

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1. Power on the computer and the light source for the instrument. Turning on the computer automatically powers the MSP. Let it stabilize for a minimum of 30 minutes.
2. Open the "CRAIC Data" and "CRAIC Image" programs.
3. In the CRAIC Data "GRAMS" window select the "Auto Calibration" tab at the bottom of the screen.
4. Select the "My Folder" button and create or select the folder that you want to the data file saved to. Select "OK".
5. Place the focusing slide located in the standards box on the stage and focus one of the red dots in the field of view.
6. Focus the field diaphragm to set proper Köhler illumination.
7. With the sampling window focused off of the red dot select the "AutoSet Optimization" button. If the "AutoSet" function passes, a smiley face will appear, select "OK".
8. Block the light to the spectrophotometer head by pulling out the rod on the upper left side of the MSP (on the right side for the operator) to "VIS" position.
9. Collect a dark scan by selecting the "Collect DarkScan" button in the "GRAMS" window.
10. Restore the light to the MSP head by pulling out the rod on the upper left side of the microspectrometer to the "QDI" position.
11. Collect a reference sample by selecting the "Collect Reference" button in the "GRAMS" window.
12. Run the wavelength calibration
    a. Select the "Auto Cal Wavelength Check" button in the "GRAMS" window.
    b. Place the holmium oxide standard filter over the field diaphragm on the base of the microscope and select "OK".
    c. Replace the holmium oxide filter with the didymium standard filter and select "OK".
    d. If the calibration passes, a smiley face will appear and the operator will be asked if they want to print the calibration certificate. The appearance of the smiley face means that the acquired values for the holmium oxide and the didymium standards are acceptable and fall within the range of acceptable NIST standard values stored in the software. Print the calibration certificate and initial the page. Retain copies of the calibration certificate in the MPD instrument log, the case file and the FBI instrument log. If the acquired values do not pass the calibration see step 14 of the Daily Performance Standard section.
13. Run the Photometric Calibration
    a. Select the "Auto Photometric Check" button in the "GRAMS" window.
    b. Place the 0.1 neutral density (ND) filter over the field diaphragm and select "OK".
    c. Replace the 0.1 ND filter with the 0.5 ND filter and select "OK".
    d. Replace the 0.5 ND filter with the 1.0 ND filter and select "OK".
    e. If the calibration passes a smiley face will appear and the operator will be asked if they want to print the calibration certificate. The appearance of the smiley face means that the acquired values for the neutral density filters are acceptable and fall within the range of acceptable NIST standard values stored in the software.

Print the calibration certificate for the neutral density filters and initial the page. Retain copies of the calibration certificate in the MPD instrument log, the case file and the FBI instrument log.

f. If the acquired values do not pass the calibration see step 14 of the Daily Performance Standard section.

14. If the acquired values for the holmium oxide, didymium or neutral density standards fall outside the accepted range, wait approximately 30 minutes and re-analyze the standards. If the values still fall outside the accepted range, the instrument will be taken out of service and appropriately labeled. A service call will be placed for the instrument.

Daily Calibration for the QDI 2010 microspectrophotometer

Verification of calibration must be performed each day the MSP system is used. Save all of the spectra in an appropriate folder on the computer. Spectral files should be stored in each examiner's folder which contains a folder with the MPD Laboratory number as the folder's name.

15. Power on the computer and the light source for the instrument. In addition, turn on the microscope (button on left), the spectrometer (button on the back right), and select the filter turret (at the base of the microscope on the right side) for transmission spectra "TL" and turn off the turret for reflectance spectra "RL". Let the light source stabilize for a minimum of 30 minutes.
16. Open the CRAIC Image Software and CRAIC MSP Data Acquisition Software programs in that order (icons on the computer desktop). Note that there is one software program (CRAIC Data Acqusition Software) for generating spectra and two software programs (CRAIC Data Acquisition Software and GRAMS AI Software) for analyzing spectra.
17. In the CRAIC Imaging window click "auto" for gain and exposure. Click "save".
18. In the CRAIC data window select the "Tools" tab in the menu bar and then select the "auto calibration/transmission" tab.
19. Select the "My Folder" button and create or select the folder that you want to save your data to. The proper folder for calibration spectra is obtained through the following sequence of folders: "C:/CRAIC Data/QC calibration verification/Calibration verification/Cal 2740/Cal 2011 or current year". Once you have selected the proper folder click the "select Cur Dir" button. Click "save".
20. Place the focusing slide, located in the standards box, on the stage and focus on one of the ink lines in the field of view.
21. Unclick "auto" on imaging window for gain and exposure.
22. Set proper Kohler illumination by focusing the edges of the field diaphragm.
23. Move the sampling window off of the ink line and select the "auto set optimization" button on the auto calibration/transmission window. If the Autoset function passes, a smiley face will appear. Select "OK".
24. Block the light to the MSP head by pushing in the eyepiece viewing rod on the upper left side (right side for operator) of the MSP.
25. Collect a dark scan by selecting the "Collect Darkscan" button at the bottom of the CRAIC window.

26. Restore the light to the MSP head by pulling out the eyepiece viewing rod.
27. Collect a reference sample by selecting "Collect Reference" button in the CRAIC data acquisition software window.
28. Select the "Wavelength Check" button.
    a. Place the Holmium Oxide standard filter over the window at the base of the microscope and select "OK".
    b. Replace the Holmium Oxide standard filter with the Didymium standard filter and select "OK".
    c. If the calibration passes, a smiley face will appear and you will be asked if you want to print the calibration certificate. The appearance of the smiley face means that the acquired values for the standards are acceptable and fall within the range of acceptable NIST standard values. Click "Print & Save", select printer, and click "OK". There is a switch that must be switched to the number 2 position to print the document. Retain copies of the calibration results in the MPD instrument log, the case file, and the FBI instrument log.
    d. If the acquired values do not pass the calibration see step 30 of this section.
29. Select the "Photometric Check" button.
    a. Place the 0.1 neutral density (ND) filter over the window at the base of the microscope and click "OK".
    b. Replace the 0.1 ND filter with the 0.5 ND filter and select "OK".
    c. Replace the 0.5 ND filter with the 1.0 ND filter and select "OK".
    d. If the calibration passes, a smiley face will appear and you will be asked if you want to print the calibration certificate. The appearance of the smiley face means that the acquired values for the ND filters are acceptable and fall within the range of acceptable NIST standard values. Click "Print & Save", select printer, and click "OK". There is a switch that must be switched to the number 2 position to print the document. Retain copies of the calibration results in the MPD instrument log, the case file, and the FBI instrument log.
    e. If the acquired values do not pass the calibration see step 30 of this section.
30. If the acquired values for the holmium oxide, didymium oxide, or ND filters fall outside of the accepted range, wait approximately 30 minutes and reanalyze the standards. If the values still fall outside the accepted range, the instrument will need to be taken out of service and appropriately labeled. A service call will be placed for the instrument.
31. Close the Auto calibration/transmission window when finished with the calibrations.

Calibration Acceptance Criteria

The holmium oxide and didymium acquired values must be within ± 3 nm of the NIST standard values and the neutral density (ND) standard values must be within the NIST specified range of standard values.

Sample Spectrum Collection for the QDI 1000 microspectrophotometer

1. In the CRAIC Data "GRAMS" window select the "Data Collection" tab at the bottom of the screen.

2. Select the "My Folder" button and create or select the folder that you want to the data file saved to. Select "Ok".
3. Place the sample slide on the stage and focus the sample in the field of view.
4. Focus the field diaphragm to set proper Köhler illumination.
5. With the sampling window focused off of the sample select the "AutoSet Optimization" button. If the "AutoSet" function passes a smiley face will appear, select "OK".
6. Block the light to the spectrophotometer head by pulling out the rod on the upper left side of the MSP (on the right side for the operator) to "VIS" position.
7. Collect a dark scan by selecting the "Collect DarkScan" button.
8. Restore the light to the MSP head by pulling out the rod on the upper left side of the MSP to the "QDI" position.
9. Collect a reference sample by selecting the "Collect Reference" button.
10. Place the sampling probe over the sample and focus the sample.
11. Focus the field diaphragm to set proper Köhler illumination.
12. Select the "Collect Sample" button in the "GRAMS" window.
    a. Enter the file name (Case number, item number, etc.).
    b. Check to make sure that the Y axis is set for absorbance.
    c. Select "OK".
    d. The spectra will appear in the data browser window.
13. Reposition the sampling probe over a different area of the fiber (if you only have one fiber) or on a different fiber (if you are working with multiple fibers as found in a known sample).
14. Repeat steps 12 and 13 until you have a minimum of 5 spectra, you may take more if you feel it is necessary.

Sample Analysis for the QDI 1000 microspectrophotometer

1. Take an average of the spectra by selecting the "AVE" button on the top of the "GRAMS" window.
    a. With the "Numbered files" radial button selected click on the "Browse" button.
    b. Type in the file name under which to save the averaged spectrum and select "Ok".
    c. Select the "Select files" radial button and then click, "Ok".
    d. Select the individual files you want to average together, click "Open".
    e. Select the "Create average" button.
2. Open the "View with legend" tab on the bottom of the "GRAMS" window.
3. The data browser window displays the files that are currently open.
    a. To remove a curve, select the file and hit the remove button.
    b. To change a curves color, size and visibility, go to "Color/Style", select the file you wish to work with, make the desired changes, and click "Ok".
4. To change the field of view select "View", "Trace limits" and enter the axis ranges you wish to be visible, and then select "OK".
5. Change the legend to view the file names by right clicking in the legend and selecting properties. Select display title information, and deselect unwanted information. Click "OK".

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6. To print the spectrum make sure to go to "File", "Page Setup" and change the setup to the landscape setting before going to "File", "Print Report". If a spectrum is printed, the MCL number(s), item number(s), date of collection, and the operator's initials must be hand written on the top of the spectrum.
7. To compare spectra from different samples:
    a. Remove all files from the data browser.
    b. Go to "File", "Open" and select the spectra you wish to view.
    c. Make any changes to the legend and field of view.
    d. Print out a report.

    Note - The examiner may include single spectra that best represent the samples collected, or mean spectra. Similar types must be compared (single spectra compared with single spectra or means compared with means). If spectra containing means are printed, the word "Average" or the letters "AVE" must be part of the text describing the spectra.
8. When you are finished running your samples, close out of the CRAIC Data and CRAIC Image windows. You will be asked if you want to save the changes to the workbook. Select "NO". Turn off the light source and shut down the computer.

Sample Spectrum Collection for the QDI 2010 microspectrophotometer

1. In the CRAIC MSP data acquisition window, select the "My Folder" button and create and/or select the folder that you want the data files saved to. Click on the "Select Cur Dir" button. Click "save".
2. Place the sample slide on the stage and focus the sample.
3. Focus the field diaphragm to set proper Kohler illumination.
4. With the sampling window off the sample, select the "Auto Set optimization"button. If the Auto Set function passes, a smiley face will appear. Click "OK".
5. Block the light to the MSP head by pushing in the eyepiece viewing rod.
6. Collect a dark scan by selecting the "Collect Darkscan" button.
7. Restore light to the MSP by pulling out the eyepiece viewing rod.
8. Collect a reference sample by clicking the "Collect Reference" button.
9. Place the sampling aperture over the sample and click "Collect Sample".
    a. Enter the file name (case number, item number, etc).
    b. Check to make sure that the Y axis is set for absorbance.
    c. Select "OK".
    d. The spectra will appear in the data browser window.
10. Reposition the sampling probe over a different area of the fiber (if you only have one fiber) or on a different fiber (if you are working with multiple fibers as found in a known sample).
11. Repeat steps 9 and 10 until you have a minimum of 5 spectra.

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Sample Analysis for the QDI 2010 microspectrophotometer

1. To average your spectra, select the "Tools" tab on the menu bar of the CRAIC data acquisition window. Select the "statistics" tab and choose the files in the proper folder that you wish to average and click "OK".
2. The file names show up for the spectra average as well as for the +/- 1 or 2 standard deviations. Click "OK".
3. The spectra are listed in the QDI 2010 filename list on the right side of the window. Double clicking on a file name opens a menu that allows editing of color, y-axis units, and file information.
4. Click on the Grams AI icon on the desktop. Click on the "Print with Legend" tab on the bottom of the window.
5. Go to "file", "open" and select the spectra that you would like to open. Only 5 spectra can be opened at a time. More spectra will need to be opened in a separate operation. The opened files will appear in the spectra legend at the bottom of the screen.
6. The data browser window also displays the files that are currently open.
    a. To remove a curve, select the file in the data browser window and click "remove".
    b. To change the color, size, or visibility of a curve, click on "Color/style", select the file you wish to change, make the desired changes, and click "OK".
7. To change the field of view go to "View" and "Trace limits" and enter the axis ranges and then select "OK".
8. Change the legend to view the file names by right clicking in the legend and selecting "properties". Select "display title information"and deselect unwanted information. Click "OK".
9. To print the spectrum make sure to go to "File, "Page Setup", and change the setup to landscape. Go to "File", "Print Report". If a spectrum is printed, the MCL number(s), item number(s), date of collection, and the operator's initials must be handwritten on the top of the spectrum.
10. To compare spectra from different samples:
    a. Remove all files from the data browser.
    b. Go to "File", "Open" and select the spectra you wish to compare.
    c. Make any changes to the legend and field of view (i.e. trace limits).
    d. Print out a report.

    Note – When conducting spectral comparisons, the examiner may include single spectra that best represent the samples collected or a mean spectra. Similar types must be compared (single vs. single, mean vs. mean). If mean spectra are printed, the word "average" or the letter "avg" or "ave" must be part of the text describing the spectra.
11. When you are finished analyzing your data, close out of the CRAIC data and CRAIC image windows. When asked if you want to save changes made to the workbook, click "NO". Turn off the light source and microscope and shut down the computer.

Sample Acceptance Criteria

1. Fiber spectra must be compared to determine if an association is present. The spectra must be plotted on the same page (either on the computer monitor or paper plot) on the same absorbance and wavelength scales. The position of the peak maxima, peak minima, peak width, and peak intensity must all be considered.
2. Fibers can be associated when their spectra are consistent in all peak maxima, peak minima, peak widths, and peak intensities.
3. Fibers can be excluded when one spectrum is significantly different than that of another spectrum with respect to peak maxima, peak minima, peak width, and peak intensity.
4. An inconclusive spectral comparison result may occur.
5. A copy of the spectra used in a fiber association will be included in the case notes.
6. A copy of a standard spectra from a spectral library that assists in the identification of the associated fiber sample should also be included in the case notes.

1.2 Instrument Repair and/or Maintenance

If the instrument has undergone repair and/or maintenance, or the instrument goes outside the control of the MPD Laboratory, a calibration verification must be performed before being used in casework. The procedure for calibration verification is described in the Daily Calibration section of the Procedure.

If following repair and/or maintenance the calibration and/or calibration verification is performed by an external source (e.g., FBI TEU), the MPD Trace Evidence Unit must verify and document who performed the verification and if the results are acceptable. In addition, a daily performance standard must be tested by the MPD Trace Evidence Unit prior to use.

Limitations

Only properly trained personnel shall perform the duties involved in the operation, maintenance or troubleshooting of this instrument.

When performing comparative fiber analysis, fiber samples must be examined in the same mounting medium. Dye identification and a determination of the number of different dyes used to color a fiber are not possible with MSP.

Comments

Not applicable.

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Documentation

The following worksheet(s) shall be generated and managed:

Documentation
Microspectrophotometer User's Log

References

- Craic QDI 1000 Microspectrophotometer, Manual
- Craic QDI 2010 Microspectrophotometer, Manual
- ASTM E 275-93, Standard Practice for Describing and Measuring Performance of Ultraviolet, Visible and Near Infrared Spectrophotometers, Philadelphia, PA. ASTM, 1993.
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- Hartshorne, A. W. and D. K. Laing., The Definition of Colour for Single Textile Fibres by Microspectrophotometry, *Forensic Sciences International*, 34, 107-129, 1987.
- Eyring, Michael D., Visible Microscopical Spectrophotometry in the Forensic Sciences. In: Forensic Science Handbook, 2nd edition, R. Saferstein (ed), Prentice-Hall, Inc., pp 321-387, 2002.
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- Forensic Examination of Fibres, J. Robertson (ed), chapter 10, 251-289, Taylor & Francis, 1999.